NEURITOCGENIC AND ENCEPHALITOCGENIC PROPERTIES OF
THE PERIPHERAL NERVE BASIC PROTEINS

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ABSTRACT

Two basic proteins, P1 of molecular weight 14,200 and P2 of molecular weight 12,300, purified from bovine peripheral nerve, were assayed for biological activity. The P1 protein is an exclusively neuritogenic agent, capable of producing clinical signs of experimental allergic neuritis (EAN) and histological abnormalities in the peripheral nervous system (PNS) of guinea pigs and rabbits, without any changes in their central nervous system (CNS). P2 protein, like the CNS basic encephalitogenic protein (BE), has combined neuritogenic and encephalitogenic activities, therefore it induces in these animals neurological signs and pathological evidence of EAN, as well as histological characteristics of experimental allergic encephalomyelitis (EAE).

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system (CNS), induced in laboratory animals by injection, in complete Freund's adjuvant (CFA), of brain or spinal cord tissue, or the basic protein isolated thereof (11, 13, 14, 25). The disease seems to be an appropriate model of acute post-infectious disseminated encephalomyelitis (ADE) in man (5). Experimental allergic neuritis (EAN) is the peripheral nervous system (PNS) counterpart of EAE, induced in animals with whole peripheral nerve homogenate or its acid extract (31, 32). EAN might possibly serve as an experimental model for idiopathic polyneuritis (Guillain-Barré syndrome, GBS) in man (1, 6). Although the pathogenesis of the PNS diseases (i.e., EAN, GBS) is almost identical to that of the CNS diseases (i.e., EAE, ADE) (2), the responsible agent in EAN and GBS has not yet been identified (28).

A few groups have attempted the isolation of PNS basic proteins and have indicated that they differ in composition and biological activity from the basic protein of the CNS. Thus Bencina et al. (8) observed that whereas injection of whole bovine sciatic nerve to guinea pigs led to lesions in the peripheral nervous system, injections of bovine PNS basic protein mixtures led to lesions in the

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This paper is dedicated to the memory of the late Dr. Yaakov London, who was tragically killed in the October War in the Middle East. Dr. London was the moving spirit in initiating this investigation.

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CNS exclusively, and not to EAN manifestations. A basic protein isolated by Paty (26) from human peripheral nerve was found to produce EAE, with very little neuritis, in guinea pigs. Tobari and coworkers (29, 30) isolated two main basic proteins from bovine PNS, but these proteins were devoid of disease-inducing activity. A basic protein fraction, denoted "Mp", that was isolated by Kiyota and Egami (20) from PNS of ox and rabbit, induced histological changes in PNS as well as CNS of rabbit. Eylar and coworkers (9, 10, 12, 16) purified a major protein denoted P₀ and two basic proteins designated P₁ and P₂ from PNS of several animal species. This P₁ protein was found to be very similar in size and amino acid composition to the brain basic encephalitogenic protein (BE), whereas the P₀ and P₂ proteins were unique to the PNS. Their P₂ induced in guinea pigs only EAE and in monkeys it induced both EAE and EAN, while rabbit PNS myelin or whole tissue induced only EAN (10). Hence, the PNS basic protein(s) capable of specifically inducing EAN have not yet been clearly defined.

London (22) purified two basic proteins from bovine sciatic nerve, which he also denoted P₁ and P₂. However, whereas this P₂ protein is similar to Eylar's P₂ protein (12), the P₁ protein he isolated differs in its molecular weight and composition from both the CNS basic encephalitogenic protein and from the P₁ protein isolated by Eylar and coworkers. This P₁ protein isolated by London is, therefore, referred to hereafter as P₁L. The aim of this investigation, which was initiated as a collaborative study with Dr. London before his tragic death, is to describe the biological properties of the two PNS basic proteins isolated by London, and their capacity to induce EAE and/or EAN.

**Materials and Methods**

**Antigens**

1. *Peripheral nerve basic proteins (P₁, P₂):* Two basic proteins, P₁ and P₂, were purified from bovine sciatic nerve according to the method described by London (22). The isolation and characterization procedures were as follows: Delipidation with chloroform-butanol mixtures, dry acetone and dry ether; Acid extraction at pH 2; Dialysis against distilled water, lyophilization and solubilization in pH 10.7 buffer; Ion exchange chromatography on QAE-Sephadex G-25 and gel filtration on Sephadex G-75. The molecular weights of the two basic proteins were found to be: P₁ = 14,200 (±600), P₂ = 12,300 (±600). The amino acid composition of the two basic proteins were similar to each other, as reported previously (22), but differed markedly from the amino acid composition reported for the basic encephalitogenic protein from central nervous system (23).

2. *Central nervous system basic encephalitogenic protein (BE):* Was prepared from bovine spinal cord by column chromatography on Sulfoethyl-Sephadex, as described previously (19). The calculated molecular weight of the BE was 18,000.

3. *Peripheral nerve homogenates (PN):* Were prepared from human, bovine, guinea pig and rabbit sciatic nerve. The nerve fibers were cut in thin sections and homogenized with an equal volume of physiological saline solution.

**Animals**

The animals used were DH albino guinea pigs weighing 250–350g, and New Zealand albino rabbits weighing 2500–3500g.
Immunization of animals

Guinea pigs were immunized with 10 μg or 100 μg P1, 10 μg or 100 μg P2, or 10 μg BE. The antigens were injected emulsified in complete Freund’s adjuvant (CFA), 0.1 ml into each hind footpad. The PN homogenates were injected with CFA in the same way, in an approximate amount of 40 mg (wet weight) of nerve tissue per animal. Rabbits were injected with 500 μg P1, 500 μg P2 or with about 300 mg rabbit PN in CFA into the footpads of the hind legs.

Symptomatology

Animals were observed daily for loss of weight and for the appearance of neurological signs. Clinical evaluation was carried out using the criteria of Hall (17): [I] Mild involvement—loss of weight, ataxia only noticeable on displacement; [II] Moderate involvement—ataxia present upon walking, abnormal position of one or both hind limbs; [III] Severe involvement—animal grossly disabled and only able to move with difficulty. Neurological signs of EAE were reflected by paralysis of the hind legs [IV].

Histology

Sciatic nerve and spinal roots, as well as the brain and the upper part of the spinal cord were examined. The animals were sacrificed after 3-4 weeks and specimens were fixed in 10% formalin for at least 3 days. Transverse and longitudinal sections were stained with luxol fast blue-kern echtrot (Luxol) hematoxylin-cosin-phosphomolybic acid-light green (H.E.).

The grading of the histopathological changes in PNS was based, according to Hall (17), on the degree of myelin degeneration and cellular infiltration in the sciatic nerve: [I] Mild—slight myelin damage confined to nodal regions and/or proliferation of Schwann cells, with or without one or two small foci of cellular infiltration; [II] Moderate—scattered, mildly or moderately sized cell infiltrations with degenerating myelin extending over one or two internodes; [III] Severe—many foci of cell infiltration, often diffuse and dense with marked area of myelin degeneration. The grading of the histopathological changes in CNS was based on the degree of cellular infiltration: [I] Mild—slight perivascular infiltration; [II] Moderate—marked perivasculitis; [III] Severe—massive perivasculitis, periventriculitis and parenchymatous cellular infiltration.

RESULTS

Peripheral nerve homogenates of human, bovine, guinea pig and rabbit origin were injected into guinea pigs. Clinical signs of EAN were observed 2–3 weeks after inoculation in 70–80% of these animals (Table I). Histopathological changes characteristic of EAN were observed in the PNS of 80–100% of these animals. Both the neurological and the histological abnormalities were more pronounced when rabbit and guinea pig PN were injected (average grade II–III) than when human or bovine PN were tested (average grade I–II). Histopathological changes in the CNS were only minimal (grade I) and were observed in a small proportion of the animals (10%) when human or bovine PN were injected, and 20–30% when guinea pig or rabbit PN were used. Rabbits injected with rabbit PN homogenate evinced a similar incidence and degree of clinical and histological signs in the PNS and showed a comparable limited extent of CNS involvement.

Purified basic proteins (P1 and P2) of bovine PNS origin were assayed for their neuritogenic and encephalitogenic properties in guinea pigs and in rabbits (Table II). Both clinical and histological signs of EAN, without any pathological
TABLE I

Incidence and Degree of EAN and EAE Induced by Peripheral Nerve Homogenates (PN)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Animal</th>
<th>Number of animals tested</th>
<th>Clinical signs</th>
<th>PNS histological lesions</th>
<th>CNS histological lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Incidence (%)</td>
<td>Range of severity*</td>
<td>Day of onset (weeks)</td>
</tr>
<tr>
<td>Human PN</td>
<td>guinea pig</td>
<td>10</td>
<td>70</td>
<td>I-II</td>
<td>15-22</td>
</tr>
<tr>
<td>Bovine PN</td>
<td>guinea pig</td>
<td>10</td>
<td>80</td>
<td>I-II</td>
<td>15-22</td>
</tr>
<tr>
<td>Guinea pig PN</td>
<td>guinea pig</td>
<td>10</td>
<td>80</td>
<td>II-III</td>
<td>14-19</td>
</tr>
<tr>
<td>Rabbit PN</td>
<td>guinea pig</td>
<td>10</td>
<td>80</td>
<td>II-III</td>
<td>14-19</td>
</tr>
<tr>
<td>Rabbit PN</td>
<td>rabbit</td>
<td>5</td>
<td>80</td>
<td>I-II</td>
<td>14-17</td>
</tr>
</tbody>
</table>

* The characterization of antigens and the method of immunization as well as the various degrees of the clinical and histological findings are described and defined under materials and methods.

TABLE II

Incidence and Degree of EAN and EAE Induced by Purified PNS and CNS Antigens

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Animal</th>
<th>Number of animals tested</th>
<th>Clinical signs</th>
<th>PNS histological lesions</th>
<th>CNS histological lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Incidence (%)</td>
<td>Range of severity*</td>
<td>Day of onset (weeks)</td>
</tr>
<tr>
<td>10 µg P1</td>
<td>guinea pig</td>
<td>10</td>
<td>10</td>
<td>I</td>
<td>14</td>
</tr>
<tr>
<td>100 µg P1</td>
<td>guinea pig</td>
<td>20</td>
<td>80</td>
<td>I-II</td>
<td>14-20</td>
</tr>
<tr>
<td>500 µg P1</td>
<td>rabbit</td>
<td>5</td>
<td>60</td>
<td>I</td>
<td>17-21</td>
</tr>
<tr>
<td>10 µg P2</td>
<td>guinea pig</td>
<td>10</td>
<td>0</td>
<td>I</td>
<td>0</td>
</tr>
<tr>
<td>100 µg P2</td>
<td>guinea pig</td>
<td>20</td>
<td>60</td>
<td>I</td>
<td>17-20</td>
</tr>
<tr>
<td>500 µg P2</td>
<td>rabbit</td>
<td>5</td>
<td>60</td>
<td>I</td>
<td>15-19</td>
</tr>
<tr>
<td>10 µg BE</td>
<td>guinea pig</td>
<td>10</td>
<td>90</td>
<td>IV</td>
<td>14-20</td>
</tr>
</tbody>
</table>

* The characterization of antigens and the method of immunization as well as the various degrees of the clinical and histological findings are described and defined under materials and methods.

evidence of EAE in the CNS, were found when P1 was given in doses of 100 µg per guinea pig or 500 µg per rabbit. Mild or moderate histological lesions (Fig. 1) were found in the sciatic nerves of 90–100% of those animals. A low incidence of clinical and pathological changes were found even when a dose of only 10 µg P1 protein was inoculated (Fig. 2).

P2 protein induced mild neurological manifestations in 60% of guinea pigs and rabbits receiving doses of 100 µg or 500 µg, respectively. Histologically, it was found that this protein caused mild to moderate (in guinea pigs) or moderate (in rabbits) CNS abnormalities (Fig. 3) in 80% of the animals, but only mild PNS abnormalities in 40% of the animals. Approximately the same PNS histological changes were found in guinea pigs after injection with 10 µg of the basic encephalitogenic protein of the brain (Fig. 4), but in this case CNS abnormali-
ties were much more pronounced (grades II–III) and most of the animals were paralyzed (grade IV). A dose of 10 µg P₂ was ineffective in induction of either clinical or histological signs.

**DISCUSSION**

In the present study we have examined the neuritogenic and encephalitogenic properties of peripheral nerve tissue homogenates (PN) and of two basic proteins, (P₁ and P₂) isolated from bovine sciatic nerve by the method of London (22). Our findings indicate that 1) the PN tissue whole homogenates have strong neuritogenic and minimal encephalitogenic activities; 2) P₁ has moderate neuritogenic properties without any encephalitogenic capacity; 3) P₂ is only mildly neuritogenic but has moderate encephalitogenic activity; it is thus similar in its biological activity to the basic protein (BE) of the CNS.

Experimental allergic neuritis has previously been reported in a wide variety
of animals (3, 4, 6, 7, 17, 18, 21, 24, 27, 31, 32) immunized with peripheral nerve tissue of various mammalian species. In most of these reports histopathological lesions were found exclusively in the peripheral nervous system (4, 6, 7, 17). However, several studies have revealed the involvement of the CNS in animals with EAN (32) and, similarly, PNS lesions have occasionally been observed in animals receiving CNS antigens (4, 15, 24, 31, 33). In the present study we confirm such findings; PNS changes were found in most guinea pigs and rabbits challenged with peripheral nerve, accompanied by minimal changes in the CNS of a small proportion of these animals (Table I). Both clinical and histological signs of EAN were more severe when guinea pig or rabbit nerve tissues were used rather than human or bovine, while the CNS involvement was similar irrespective of the origin of the peripheral nerve tissue. In 50% of the animals challenged with CNS basic encephalitogenic protein (BE) mild PNS ab-
normalities were found in addition to the prominent signs of EAE (Table II). These results indicate diverse susceptibility of the CNS and the PNS to disease induction by peripheral nerve tissue of various species, and provide preliminary evidence of a common component of peripheral and central nervous system tissues. Indeed in vitro evidence for a common antigenic component in extracts of PNS and CNS has also been reported (6, 26, 28).

The results described hitherto concern studies with whole tissue homogenates or extracts. Investigations carried out with isolated peripheral nerve proteins point to a similar phenomenon, namely, the capacity of a single component to induce both EAN and EAE. Based on these observations it has been postulated that two different sites exist on the same protein. Thus, Kiyota and Egami (20) suggested that two factors are present in the “Mp” component, a “demyelinating factor” specific to PNS and a “promoting factor” which causes infiltrations in both CNS and PNS. Similarly, Eylar and his colleagues (10) proposed the hypothesis that different disease-inducing sites exist in their P₂ protein one induces EAE, and the other induces EAN.

The P₂ protein prepared according to London (22), which was used in the present study, is similar to the P₂ protein described by Eylar et al. (12), in its chemical and immunological properties. When this purified protein was injected into guinea pigs and rabbits, it induced both EAE and EAN (Table II), in agreement with the above-mentioned theory concerning its encephalitogenic and neuritogenic activities. The mild neuritic changes seen in guinea pigs after challenge with P₂ in our study, as well as the essential absence of neuritic
changes in other studies where related protein were given (8, 10, 26), could be explained either by a weak neuritogenic determinant on these proteins or by the small amount of the target P$_2$ protein in guinea pig peripheral nerve myelin (16). As discussed above, the incidence and degree of encephalitis seen in animals injected with whole peripheral nerve is minimal. This phenomenon could be explained, as suggested previously (10), by a presumed block of the P$_2$ protein or its encephalitogenic site in the native myelin structure. Generally, the PNS basic P$_2$ protein has similar encephalitogenic and neuritogenic properties to those of the CNS basic protein although the latter is apparently more active.

The results obtained with P$_1$ protein are unique and do not correspond to any data reported previously. This protein is purely neuritogenic and does not induce any histopathological changes in the CNS (Table II). These findings may be due to the fact that P$_1$ protein is different from other proteins tested in parallel studies. Thus, it differs in its molecular weight, amino acid composition and
biological activity from both the CNS basic protein and from the PNS basic P₁ protein described by Eylar and coworkers (9, 16), as well as from the P₂ protein. The possibility has been raised that P₂ protein is derived from P₁ as a result of proteolytic activity in situ (9). However, the differences in amino acid composition and in the biological activities of these P₁ and P₂ proteins would be difficult to reconcile with this idea.

In conclusion, the results of this study corroborate previous observations that components of the peripheral nervous system are both neuritogenic and encephalitogenic; the same is true for the purified P₂ protein. It is also clearly demonstrated, for the first time, that at least one of the PNS proteins, P₁, has pure neuritogenic activity.

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